Synaptic Transmission Is Delayed During NREM Sleep Compared to Awake State in the Mouse Hippocampal CA1 Commissural Pathway.

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Abstract

The hippocampus is essential for spatial learning and memory, and sleep plays a major role in memory processing. In this study, using in vivo electrophysiological methods, we characterize the latency of hippocampal CA1 field Excitatory Postsynaptic Potentials (fEPSP) response. Then, we compared the latency during Non-rapid eye movement (NREM) sleep and the awake state during the period after fear-conditioning learning. We observed that after learning, the synaptic response during NREM state was delayed by about 0.3 ms compared to awake state. These results suggest that the speed of synaptic transmission is different between the NREM and the awake state. This effect could potentially come from the differences in neurochemical composition of the CA1 region during the NREM and the awake state.

Introduction

The hippocampus plays a crucial role in long term memory formation and learning. The hippocampus along with the medial temporal lobe is responsible for storing new declarative memory (Frankland et al, 2005). Non-rapid eye movement (NREM) sleep has been implicated in the consolidation of hippocampus-dependent declarative memories (Mölle et al, 2004). To form a better understanding of the role of sleep in hippocampal memory consolidation, we investigated hippocampal CA1 region fEPSP response latency during post-learning NREM sleep state and awake state using in vivo electrophysiology.

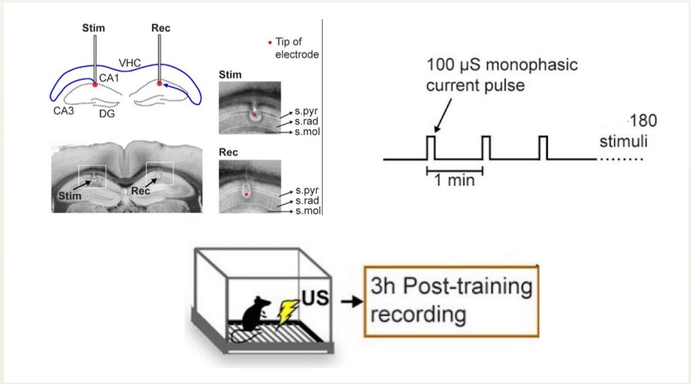

Figure 1. Experimental setup for recording. Top Left: Schematic drawing showing the location of the stimulating (stim) and the recording (rec) electrodes, and the CA1-to-CA1 commissural pathway (blue) coursing through the ventral hippocampal commissure (VHC). Top Right: Stimulation protocol applied to the left CA1, which was stimulated every 1 min with 100 µs square pulses for 3h. Bottom: Cartoon showing the fear-conditioning learning (US - unconditioned stimulus) followed by 3h recording. (Diagrams adapted from Subramaniyan et al 2020).

Methods and Materials

To study the commissural pathway that connects the left and right hippocampal CA1 regions, we inserted a stimulating electrode in the left hippocampus and the recording tetrodes (1-7) in the right hippocampus CA1. We gave electrical pulses every minute for 3 hours after a fear conditioning learning event. We also monitored the sleep state of the mice during the post-learning period. After the data were collected, we averaged the stimulation responses and computed the response latency for NREM and awake state. We excluded mice that had post-learning sleep that was less than 5 minutes. All data analyses and statistics were done using custom-written code in MATLAB.

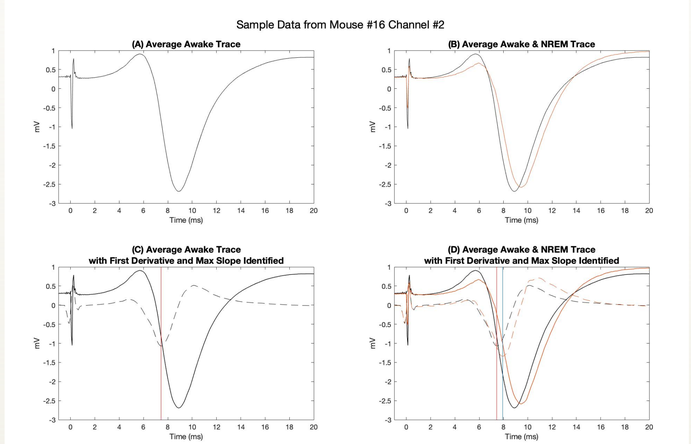

Figure 2. Single recording channel example showing the computation of fEPSP latency. (A) The fEPSP response trace averaged across pulses during the awake state. (B) Average fEPSP response trace during the awake (black) and NREM state (orange). (C) Average fEPSP response trace of awake state (black) and its first derivative (dotted line). The latency of the response is taken as the time of trough (red) of the derivative (the maximal slope of the response). (D) Same as C except NREM sleep response trace (orange) is also included. The averaged traces are z-scored.

Results

We analyzed the data by calculating the maximal slope value of average fEPSP traces of each channel by each mouse of sleep and awake state as demonstrated. For mice with multiple recording channels, the results were calculated separately by each channel and then averaged across all channels. We observed that neuronal transmission in the CA1 region has 0.28 ± 0.048 ms (mean ± 1 standard error) longer latency in NREM sleep state than the awake state ($p = 0.000019$, $n = 19$, paired t-test).

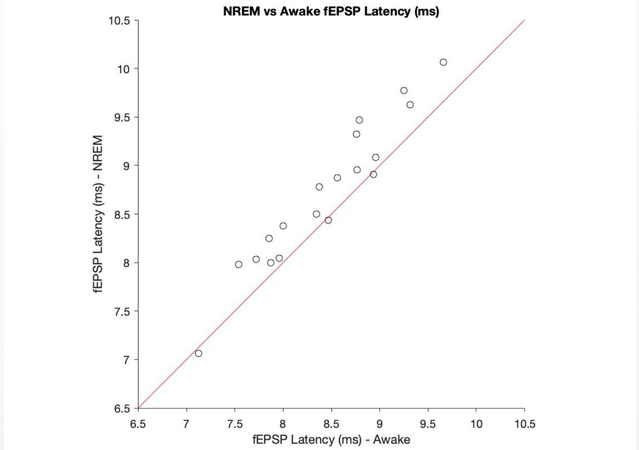

Figure 3. NREM vs Awake State fEPSP latency. Each data point represents a mouse. Since almost all the data points appear above the equality line (red line), fEPSP latency is longer during the NREM sleep.

Discussion

The result is interesting because the delayed latency in CA1 neuronal representation in post-learning sleep may be important for sleep-dependent memory consolidation. However, due to the lack of the control condition on the NREM and awake state fEPSP latency with no learning, it is unclear if the delayed latency is necessary for learning. Future studies will address this by measuring the latency effect before and after learning.

Acetylcholine, Norepinephrine and serotonin levels are low in the hippocampus during NREM sleep compared to awake state (Watson et al, 2010). This difference in the neuromodulators could potentially explain the latency effect we observed. However further studies are needed to investigate how these neurotransmitters may bring this effect.

Synaptic conduction may be another explanation for the delayed latency effect observed. The stimulus pulse is passed from the left hemisphere to the right hemisphere (recording sites) through the commissural pathway. During sleep and awake states, different pathways involving the commissural pathway are active (Neves et al, 2008). Such differences can lead to differences in signal transmission rate presynaptic to the recording site. For the same reason, different circuits for postsynaptic modulation of the recording sites can also cause the discrepancy. Further investigation is required to identify the responsible circuits.

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